REMARKS

In view of the above amendments and the following remarks, reconsideration of the outstanding office action is respectfully requested.

Pursuant to 37 CFR § 1.21, attached as an Appendix is a Version With Markings to Show Changes Made to the amended claims.

Enclosed herewith are copies of the non-patent references and international patent references which were submitted to or cited by the U.S. Patent and Trademark Office ("PTO") in parent U.S. Patent Application Serial No. 09/120,663, filed July 22, 1998, now U.S. Patent No. 6,228,644. These references were cited in the Information Disclosure Statement submitted at the time of filing the present application. A total of 136 references are enclosed herewith (i.e., references 11-17, 28-122, 125-134, and 137-160 as listed on the previously submitted form PTO-1449).

The rejection of claim 17, 19, and 38-39 under 35 U.S.C. § 112 (first paragraph) as lacking enablement is respectfully traversed.

Claim 17 presently recites, in part, that the isolated hypersensitive response eliciting protein or polypeptide can be "a protein or polypeptide having an amino acid sequence encoded by a nucleic acid whose complement hybridizes, at 65°C in a medium which includes about 1M NaCl, to a DNA molecule comprising a nucleotide sequence of SEQ. ID. Nos. 1 or 3." Written descriptive support for the newly introduced limitation defining the hybridization conditions is found at page 18, lines 3-6 of this application.

The PTO has taken the position at pages 3-4 of the outstanding office action that the present application identifies nothing other than the sequences of DspE and DspF, having failed to define any "structural characteristics that would serve to identity (sic) other homologous proteins from any other species or genus" or "suggest[] the sources for other proteins that may be encompassed by the broadly recited claims." Applicants respectfully disagree.

Because the DspE and DspF proteins identified in the present application are present in a two-gene operon which lies downstream of the *hrpN* gene in *Erwinia amylovora* (strain Ea321) and presence of the operon is required for pathogenicity, the operon has been referred to as a "disease specific" (dsp) region (see Examples 8-9, pages 37-38 of this

application). Thus, one of ordinary skill in the art would be directed to identify sequences from other pathogenic bacteria (preferably though not exclusively plant pathogenic bacteria) which hybridize to the nucleic acid molecules of SEQ. ID. Nos. 1 and 3.

As for actually performing such hybridization procedures, applicants submit that one of ordinary skill in the art is readily able to utilize DNA of SEQ. ID. No. 1 or 3 (or their complements) as a probe to identify nucleic acids (DNA or RNA) which hybridize thereto. For example, a Southern hybridization procedure can be performed using DNA of SEQ. ID. Nos. 1 or 3 to probe DNA obtained from other pathogenic bacteria (Southern, "Detection of Specific Sequences Among DNA Fragments Separated by Gel Electrophoresis," J. Mol. Biol., 98:503-517 (1975)(copy attached hereto as Exhibit A); Sambrook et al., Molecular Cloning: A Laboratory Manual, 2d ed., pp. 9.31-9.58 (1989) (copy attached hereto as Exhibit B)). Upon identifying a nucleic acid molecule which hybridizes to the probe, the nucleic acid molecule can be isolated and sequenced using standard procedures to identify any open reading frames. If necessary (i.e., where the sequenced clone only contains a partial reading frame), primers can be designed based on the partial sequence to expand the size of subsequent clones, via polymerase chain reaction or alternative amplification procedures which involve chromosome walking, to encompass an entire open reading frame (Sambrook et al., Molecular Cloning: A Laboratory Manual, 2d ed., pp. 14.12-14.13 (1989) (copy attached hereto as Exhibit C)). Once obtained, the entire open reading frame can then be expressed using a recombinant expression system (i.e., plasmid vector in *E. coli*) and the expressed protein can be isolated and purified using conventional procedures (see page 18, line 26 to page 22, line 8 of this application). Moreover, based upon the nucleotide sequence, the amino acid sequence of the protein or polypeptide can be predicted. The predicted amino acid sequence can be compared to the amino acid sequence of DspE and/or DspF to determine their similarity and/or identity as measured, for example, with conventional sequence analysis algorithms, such as ClustalW, BLAST, etc. Thus, one of ordinary skill in the art is fully capable of carrying out a hybridization procedure under the conditions recited in claim 17.

Contrary to the PTO's assertion (noted above), the present application identifies a number of properties of DspE and DspF, thereby allowing one of ordinary skill in the art to compare the properties of a protein, encoded by nucleic acid whose complement hybridizes to SEQ. ID. No. 1 or 3, to DspE or DspF.

DspF is described as having a predicted molecule weight of about 16 kD, a pI of 4.45, and being predominantly α-helical with amphipathic α helices in its C-terminus (see page 38, lines 18-22). These characteristics comport with chaperone proteins of pathogenic bacteria, as reported in Wattiau et al., "Customized Secretion Chaperones in Pathogenic Bacteria," Mol. Microbiol. 20:255-62 (1996) (reporting chaperone properties as acidic pI, a size in the range of 15-20 kDa, and a putative amphipathic α helix in its C-terminal region) (copy attached hereto as Exhibit D), which is incorporated by reference into the present application. Thus, one of ordinary skill in the art would readily be able to determine whether a protein or polypeptide, encoded by a nucleic acid whose complement hybridizes to SEQ. ID. No. 3, is structurally similar to DspF of *Erwinia amylovora*.

DspE is described as a hydrophilic protein having a predicted molecular weight of about 198 kDa (see page 38, lines 14-16 of this application) and an N-terminus which includes a stable domain (see page 38, lines 16-18 and 30-32 of this application). It was demonstrated to function as an avirulence protein which is expressed in a hrp-regulated manner (see Example 11, pages 39-40, and Example 13, pages 40-41 of this application). As a result of its properties as an avirulence protein, DspE expression quantitatively effects hypersensitive response elicitation by *Erwinia amylovora* in tobacco (see Example 10, page 39 of this application) and triggers the hypersensitive response in soybeans when expressed by *Pseudomonas syringae* pv. glycinea (see Example 13, pages 40-41 of this application). Thus, one of ordinary skill in the art would readily be able to determine whether a protein or polypeptide, encoded by a nucleic acid whose complement hybridizes to SEQ. ID. No. 1, is structurally and/or functionally similar to DspE of *Erwinia amylovora*.

Finally, one of ordinary skill in the art can also consider whether the nucleic acids which encode such homologous proteins or polypeptides (i.e., whose complements hybridize to SEQ. ID. No. 1 and 3) are encoded by a single operon. DspE and DspF are encoded by a single operon (see Example 8, pages 37-38 of this application). Similarly, their homologs AvrE and AvrF of *Pseudomonas synringae* pv. tomato are encoded by genes located within a single operon, designated the *AvrE* locus (see Example 12, page 40 of this application). Thus, one of ordinary skill in the art also would expect other avirulence and chaperone proteins or polypeptides within the scope of claim 17 to be encoded by a single operon.

In view of the above amendments to claim 17, applicants submit that the present application fully enables one of ordinary skill in the art to practice the presently claimed invention.

The rejection of claims 17, 19, and 38 under 35 U.S.C. § 102(b) as anticipated by He et al., "*Pseudomonas syringae* pv. *syringae* $Harpin_{Pss}$: A Protein That Is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255-1266 (1993)("He") is respectfully traversed.

He reports the nucleotide sequence of a fragment of the *Pseudomonas syringae* pv. *syringae hrpZ* gene that encodes the hypersensitive response elicitor $harpin_{Pss}$. He also reports the amino acid sequence of $harpin_{Pss}$.

$Harpin_{Pss}$ contains 341 amino acid residues (He, Figure 5). Therefore, $harpin_{Pss}$ clearly possess an amino acid sequence which is different from the amino acid sequences of SEQ ID. Nos. 2 and 4.

Applicants also submit that the DNA sequence of SEQ ID Nos. 1 and 3 would not hybridize to the complement of a nucleic acid encoding $harpin_{Pss}$ under hybridization conditions where hybridization occurs "at 65°C in a medium which includes about 1M NaCl". As evidence that such hybridization would not occur, attached hereto are copies of sequence alignments between *dspE* and *hrpZ* (copies attached hereto as Exhibits E and F) and *dspF* and *hrpZ* (copies attached hereto as Exhibits G and H). One alignment for each was performed using the ClustalW sequence alignment tool set on its default settings (Exhibits E and G), whereas the other sequence alignment for each was performed using the Martinez/Needleman-Wunsch DNA alignment of DNAStar set on its default settings (Exhibits F and H).

As shown in Exhibit E, overall sequence homology between *dspE* and *hrpZ* was 27 percent at the nucleotide level. The alignment between *dspE* (SEQ. ID. No. 1) and *hrpZ* indicates that at most 10 consecutive nucleotides would be available for hybridization between *dspE* (SEQ. ID. No. 1) and the complement of *hrpZ* (Exhibit F). As shown in Exhibit G, overall sequence homology between *dspF* and *hrpZ* was 26.4 percent at the nucleotide level. The alignment between *dspF* (SEQ. ID. No. 3) and *hrpZ* indicates that at most 9 consecutive nucleotides would be available for hybridization between *dspF* (SEQ. ID. No. 3) and the complement of *hrpZ* (Exhibit H). Under the hybridization conditions recited

in claim 17, therefore, applicants submit He fails to teach or suggest a nucleic acid whose complement would hybridize to either SEQ. ID. Nos. 1 or 3.

For these reasons, the rejection of claims 17, 19, and 38 as anticipated by He is improper and should be withdrawn.

The rejection of claim 39 under 35 U.S.C. § 103(a) for obviousness over He is respectfully traversed. As noted above, He fails to teach or suggest a protein or polypeptide as recited in claim 17. Therefore, claim 39, which ultimately depends on claim 17, cannot have been rendered obvious over He. For this reason, the rejection of claim 39 for obviousness over He is improper and should be withdrawn.

As noted in the outstanding office action (at page 2), the PTO indicated that claims 20-28 (i.e., non-elected Groups II and III) would be rejoined with claims 17-19 and 38-39 (i.e., elected Group I) "at such time that the recited method claims read only upon allowed subject matter...." Because the presently claimed subject matter of claims 17-19 and 38-39 is allowable for the reasons noted above and claims 20-28 ultimately depend from claim 17, applicants respectfully request that the restriction requirement between Group I and Groups II and III be withdrawn in its entirety.

In view of the all of the foregoing, applicants submit that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

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11/30/01
Date

Wendy L. Harrold

Appendix

Version With Markings to Show Changes Made

In reference to the amendments made herein to claim 17, additions appear as underlined text, while deletions appear as bracketed text, as indicated below:

In The Claims:

17. (Amended) An isolated hypersensitive response eliciting protein or polypeptide selected from the group consisting of a protein or polypeptide having an amino acid sequence comprising SEQ. ID. Nos. 2 or 4, and a protein or polypeptide having an amino acid sequence encoded by a nucleic acid [which] whose complement hybridizes, at 65°C in a medium which includes about 1M NaCl, to a DNA molecule comprising a nucleotide sequence of SEQ. ID. Nos. 1 or 3.